Expression of Ki67 was 11% when 1/3 of the cells were spared but augmented till 36% when only 10% were spared.

The viability ranged between 93-98% with the highest in the 1/10 subcultivation group and the lowest in the 1/3 subcultivation group.

**Conclusion:** We could thus demonstrate a remarkable ability of this cell line to adapt to changing conditions. A decrease in doubling time up to 50 % due to reducing number of cells in each cell culture dish at subcultivation was seen. This accelerated proliferation seems to mainly have been accomplished by recruiting a higher fraction of cells in proliferation reflected by the increased Ki67 expression.

An accelerated proliferation might be of importance clinically, for example considering the effect of fractionated irradiation in a radiosensitive cell population.

**P2-123** **BSTB: Tumor and Cell Biology Posters, Tue, Sept 4**

**Induction of apoptosis by rhein via reactive oxygen species production, GADD153 expression, and caspase-3 activation in human lung cancer A-549 cells**

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**Background:** Although rhein had been shown to induce apoptosis in several cancer cell lines, the action mechanism of rhein induced cell cycle arrest and apoptosis at the molecular level is not well known.

**Method:** In this study, we use flow cytometry analysis of DNA content for cell cycle and apoptosis from A-549 cells treated with different concentrations of rhein; Poly (ADP ribose) monoclonal antibody assay for apoptosis in A-549 cells after treated with rhein; inhibition of rhein-induced apoptosis by the caspase-3 inhibitor Ac-DEVD-CHO in A-549 cells etc. We investigated the mechanisms of rhein on a human lung cancer A-549 cell that induce G0/G1 phase arrest and ROS and Ca2+ productions that play an important role for apoptosis which are characterized by caspase activation and mitochondria dependent pathway. Rhein induced G0/G1 arrest through inhibition of cyclin D3, Cdk4, Cdk4, and Cyclin E.

**Result:** The efficacious induction of apoptosis was observed at 50 μM for 12 h and up to 72 h examinations which were examined by flow cytometric method. Flow cytometric analysis demonstrated that rhein induced the loss of mitochondrial membrane potential (Δ m), cytochrome c release from mitochondrion, promoted capases-9 activation then promoted the activation of caspase-3 and led to apoptosis. Rhein also increased the levels of Gadd153, p53, p21, Bax and cytochrome c but decreased the levels of Bcl-2. The Ca2+ chelator BAPTA was added to the cells before rhein was added to the cells, and it blocked the Ca2+ production and also inhibited rhein-induced apoptosis in A-549 cells.

**Conclusions:** Our data demonstrated that rhein induces apoptosis in A-549 cells via a Ca2+-dependent mitochondrial death pathway.

**P2-124** **BSTB: Tumor and Cell Biology Posters, Tue, Sept 4**

**Focal adhesion paxillin induces nodular cell growth, invasion, and angiogenesis in lung cancer**

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Lung cancer is characterized by abnormal cell growth, invasion, and angiogenesis. The actin cytoskeleton plays a major role in these processes. In lung cancer tissues, but not other tumors, we have found that paxillin can be overexpressed, amplified, or mutated. In vivo properties of mutant paxillin (A127T) and wild type paxillin-expressing cells in nude mice were determined utilizing H522 NSCLC cell line in a mouse xenograft model.H522 has no significant expression of paxillin,therefore the properties of paxillin-transfected cells could be studied. Tumor growth in the A127T mutant paxillin markedly exceeded ($P = 0.0019$) that in the control vector or wild-type paxillin. H522 cells grew in nude mice as a solid mass without any invasion or angiogenesis, whereas both wild-type paxillin and the A127T paxillin mutant expressing H522 cells grew as nodular tumors. In addition to nodularity, A127T paxillin expressing H522 xenograft tumors were highly invasive into the adjacent muscle tissue. Upon gross examination, the A127T paxillin tumors had larger nodules as compared to the wild-type paxillin tumors. Tumor sections from mice in various groups were examined using antibodies specific for nuclear antigen Ki-67 (a marker for active cell division), CD31 (measuring microvessel density, MVD) and VEGF (vascular endothelial growth factor). There was enhanced cell proliferation, increased stroma, increased MVD and increased VEGF expression in wild type paxillin H522 xenografts. In the A127T paxillin H522 xenografts, as compared to paxillin negative or wild-type paxillin positive cells, there was enhanced cell proliferation with decreased stroma formation, MVD, and VEGF. There was a strong correlation between paxillin expression and angiogenesis ($p=0.01$). In conclusion, results from this study establish an important role for paxillin in lung cancer.

**P2-125** **BSTB: Tumor and Cell Biology Posters, Tue, Sept 4**

**Amplification of c-Met in a subset of lung cancer cells leads to activation of m-Tor pathway**

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Non-small cell lung cancer (NSCLC) is a difficult disease to treat. Even with the best therapies and the recent advent of novel molecularly targeted therapies, the overall survival for all NSCLC patients is only 20% over a five year period. c-Met receptor tyrosine kinases (RTKs) have been shown to be important in a variety of malignancies. We have examined the expression and gene amplification of c-Met receptor in various NSCLC cell lines using standard immunoblotting and FISH analysis respectively. It was found that there was overexpression of c-Met in most of the NSCLC cell lines (~75%), including H441, SKLU-1, H1993, A549, H1838, H358 and SW1573 except H-522 and H661. This was also evident in the lung tumor tissue immunoblotting and paraffin embedded lung cancer tissue micro array. FISH analysis revealed amplification of the c-Met region on chromosome 7p11.2 to copy number 15 in 22% (two out of nine) of NSCLC cell lines, and correlated with high expression. Constitutively activated c-Met was